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ANALYSIS OF INVESTIGATIONAL DRUGS IN BIOLOGICAL FLUIDS  
METHOD DEVELOPMENT AND ROUTINE ASSAY

Annual Report

April 12, 1988

Emil T. Lin, Ph.D.

Supported by:

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND  
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19. ABSTRACT (Continue on reverse if necessary and identify by block number)  This report describes the status of method development and routine analytical work under contract DAMD17-86-C-6150 at the end of the reporting period, March 15, 1987 to March 14, 1988. The purpose of work under this contract is to develop and routinely use analytical methods for the determination of the concentration in biological specimens of investigational drugs in support of pharmacokinetic and bioavailability studies undertaken for the purpose of new drug development for the US military establishment. Accepted scientific procedures including normal and reversed phase high-performance liquid chromatographic methods, post column derivatization, and protein precipitation and cartridge elution sample clean up procedures were employed in development and routine work. Methodologies for six drugs (for the assay of samples for WR 2721 in plasma, WR 3689 in plasma, pyridostigmine in urine, mefloquine in plasma, physostigmine in plasma and WR 6026 in blood) were under development, two routine analyses (for determination of the concentrations of mefloquine in plasma and pyridostigmine in plasma and urine) have been performed in support of bioavailability and pharmacokinetic studies, and routine analyses (for pyridostigmine in dog plasma and pyridostigmine in human plasma) are in progress in support of other studies. Significant progress has been made in meeting the objectives of the contract.					
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## SUMMARY

This report describes the status of work under contract DAMD17-86-C-6150 at the end of the reporting period, March 15, 1987 to March 14, 1988. Analytical methodologies for the candidate anti-irradiation chemicals, WR 2721 and WR 3689 in plasma; the anti-malaria drugs, mefloquine in plasma and halofantrine in plasma; the anti-cholinesterase drugs, pyridostigmine in urine and physostigmine in plasma; and the antileishmanial compound, WR 6026 in blood and plasma; were under development in this laboratory under the contract. Study reports were submitted for three methods. Routine analyses of samples for four studies were begun for determination of the mefloquine concentration in plasma, the pyridostigmine concentration in plasma, urine and infusates, the pyridostigmine concentration in dog plasma and the pyridostigmine concentration in human plasma. Analysis reports were submitted for two studies.



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## INTRODUCTION

The laboratory has contracted (contract number DAMD17-86-C-6150) with the US military establishment to develop and use analytical methods for detection and quantitation of candidate chemical warfare antidotes, radioprotectants and anti-infectious disease drugs to support pharmacokinetic and bioavailability studies for the purpose of new drug development. While the contracts' work statement estimated that the standard workload would include two new compounds per year for method development and characterization and 10,000 specimens for routine analysis, the laboratory during the reporting period March 15, 1987 to March 14, 1988 completed development of methodologies for three drugs (mefloquine in plasma, pyridostigmine in urine and physostigmine in plasma plus a revised halofantrine stability study), continued development of methodologies for four drugs (WR 2721, WR 3689, WR 6026 in blood to 10 ng/ml and in plasma to 1 ng/ml, and physostigmine in plasma to 0.1 ng/ml), began development of methodologies for three compounds (eseroline in plasma, WR 160, 972-mefloquines' carboxyl metabolite, and WR 225, 591-the thiol of WR 3689) and ran routine analyses on 1,375 specimens in support of two studies.

Reporting instructions required the completion of four quarterly project status and funds expenditure reports, one annual report, three study reports, and two routine analysis reports. In addition, revision of selected reports from this and previous contracts were required. Previous related submittals and revisions that were released during the period of this report were issued for contracts DAMD17-83-C-3004 and DAMD17-85-D-0008, as detailed in Table 1.

No great difficulties are foreseen in completing methods development or performing routine analyses for the drugs and studies currently targeted. Increases in the workload past current levels, however, would require the hiring of additional personnel. In addition, indirect costs to the University were increased from 32.6 to 37%. Thus, additional funding is being requested through the University of California contracting office. The technical approach that is being used in the development process is to adapt high-performance liquid chromatographic methods to the analysis of specimens for each of the compounds of interest.

## DISCUSSION

The technical work performed during the second year of the contract involved using accepted scientific procedures combined with the equipment and facilities of the University to obtain the data and to proceed with the development of methodologies as detailed elsewhere in this report. Accepted scientific procedures including normal and reversed phase high-performance liquid chromatographic methods, post column derivatization, and protein precipitation and cartridge elution sample clean up procedures were employed in development and routine work. Tables 2 and 3 list the five chromatographic systems committed to development of methodologies, each complete with a Waters Intelligent Sample

**TABLE 1: PREVIOUS RELATED SUBMITTALS**

Contract Number	Report Type Number	Drug and Specimen Type	Date of Report
DAMD17-83-C-3004	Study 1	WR 6026 in plasma	Aug. 26, 83
	Study 3	Pyridostigmine (silica gel) in plasma	Jan. 22, 85
	Study 5	Pyridostigmine (reversed phase) in plasma	Jul. 21, 86
	Study 4	Halofantrine in plasma	Aug. 23, 85
Unknown	Battelle Rat Study	Pyridostigmine in plasma	1985
	Battelle Rat Study (revised letter report)	Pyridostigmine in plasma	July 28, 87
	Battelle Dosing Sol'ns	Pyridostigmine in plasma	1985
	Battelle Dosing Sol'ns (revised letter report)	Pyridostigmine in plasma	July 28, 87
DAMD17-85-D-0008	Analysis PY85-1	Pyridostigmine in plasma (Subjects 1-30)	Aug. 11, 86
	Analysis PY85-1	Pyridostigmine in plasma (Subjects 1-30)	Sept.23, 86
	Analysis PY85-1	Pyridostigmine in plasma (Subjects 1-30)	Dec. 03, 86
	Analysis PY85-2-2	Pyridostigmine in plasma (Battelle)	Dec. 09, 85
	Analysis PY85-2-2B	Pyridostigmine in plasma (Battelle)	Jul 30, 87
	Analysis PY85-2-3	Pyridostigmine in plasma (Battelle)	Jun. 04, 86
	Analysis PY85-3-1,2	Pyridostigmine in plasma (Letterman)	Dec. 09,85
	Analysis PY85-3-3	Pyridostigmine in plasma (Letterman)	Jul. 15, 86
	Analysis PY85-3-4	Pyridostigmine in plasma (Letterman)	Mar. 03,86
	Analysis PY85-3-5	Pyridostigmine in plasma (Letterman)	Sept. 05, 86
	Analysis PY85-3-6	PY85-3-1 through PY85-3-5 combined	Apr. 20, 87
	Analysis PY85-3-6B	PY85-3-1 through PY85-3-5 combined	May. 29, 87

**TABLE 1: PREVIOUS RELATED SUBMITTALS (CONTINUED)**

Contract Number	Report Type Number	Drug and Specimen Type	Date of Report
	Analysis PY85-4	Pyridostigmine in plasma (Israel)	Aug. 12, 85
	Analysis PY85-5	Pyridostigmine in plasma (Huntingdon dog)	1985
	Analysis PY85-5B	Pyridostigmine in plasma (Huntingdon dog)	Aug. 07, 87
	Analysis PY85-5C	Pyridostigmine in plasma (Huntingdon dog)	Oct. 07, 87
	Analysis PY85-5-3	Pyridostigmine in plasma (Huntingdon dog)	1985
	Analysis PY85-5-3B	Pyridostigmine in plasma (Huntingdon dog)	Aug. 07, 87
	Analysis PY85-5-3C	Pyridostigmine in plasma (Huntingdon dog)	Sep. 30, 87
	Analysis PY85-6-2	Pyridostigmine in plasma (Johns Hopkins,Sub.4-6)	May 13, 86
	Analysis PY85-6-3	Pyridostigmine in plasma (Johns Hopkins,Sub.7-24)	Jun. 04, 86
	Analysis PY85-6-4	Pyridostigmine in plasma (Johns Hopkins,Millers)	Jul. 03, 86
	Analysis PY85-6-5	Pyridostigmine in plasma (Johns Hopkins,Sub.1-6)	Jan. 12, 86
	Analysis PY85-6-6	Pyridostigmine in plasma (Johns Hopkins,Sub.1-24)	Jan. 27, 87
	Analysis PY85-6-6B	Pyridostigmine in plasma (Johns Hopkins,Sub.1-24)	Mar. 12, 87
DAMD17-86-C-6150	Analysis AY86-1A	Halofantrine in plasma	Sep. 26, 86
	Analysis AY86-1B	Halofantrine in plasma	Jun. 11, 87
	Analysis AY86-1C	Halofantrine in plasma	Aug. 13, 87
	Analysis AY86-1D	Halofantrine in plasma	Oct. 23, 87
	Analysis AY86-2A	WR 6026 in plasma	Oct. 29, 87
	Analysis AY86-2B	WR 6026 in plasma	Feb. 12, 87
	Analysis AY86-2C	WR 6026 in plasma	Apr. 23, 87
	Analysis AY86-2D	WR 6026 in plasma	Jun. 24, 87
	Analysis AY86-3	Pyridostigmine in urine	Jan 27, 87
	Analysis Pyr/U 86-3B (revision of AY86-3)	Pyridostigmine in urine	Feb. 3, 88

**TABLE 2: CHROMATOGRAPHIC SYSTEMS COMMITTED TO DEVELOPMENT**

System	Pump	Detector
1	Varian 5000 gradient pump	Perkin Elmer 650-10S fluorescent detector
2	Beckman 110A	Perkin Elmer 204-A fluorescent detector
3	Waters 6000	Kratos Spectroflow 773 variable wavelength UV detector
4	Waters 6000	Kratos Spectroflow 773 variable wavelength UV detector
5	Beckman 100	Bioanalytical LC-4B electrochemical detector

**TABLE 3: CHROMATOGRAPHIC SYSTEMS COMMITTED TO ROUTINE ANALYSIS**

System	Pump	Detector	Attachment
1	Beckman 110A	Kratos Spectroflow 773 variable wavelength UV detector	WISP 710B and Integrator
2	Beckman 110A	Kratos Spectroflow 773 variable wavelength UV detector	WISP 710B and Integrator
3	Beckman 110A	Kratos Spectroflow 773 variable wavelength UV detector	WISP 710B and Integrator
4	Perkin Elmer series 3	Perkin Elmer 65 T variable wavelength UV detector with temperature controlled oven	WISP 710B and Integrator
5	Beckman 110B	Perkin Elmer 650-S fluorescent detector	WISP 710B and Integrator
6	Beckman 110B	Perkin Elmer 204-S fluorescent detector	WISP 710B and Integrator
7	Beckman 110B	Perkin Elmer 203 fluorescent detector	WISP 710B and Integrator
8	Varian 8500	Shimadzu RF-530 fluorescent detector	
9	Varian 8500	Shimadzu RF-530 fluorescent detector	

Processor (WISP) and a Hewlett Packard Integrator 3392A, and the nine chromatographic systems committed to the analysis of submitted specimens.

In addition, the development laboratory is equipped with three general purpose fume hoods, clean room facilities, three lockable explosion proof refrigerators, three

-20°C and two -80°C freezers, one drug cabinet, two fire proof solvent cabinets and a 60 sq. ft. solvent room. The routine analysis laboratory is equipped with two fume hoods, two explosion proof refrigerators, two -20°C freezers, one -80°C freezer, and one fire proof solvent cabinet. These and all the usual laboratory equipment (e.g., balances, pH meters, incubators, centrifuges, pipets, etc.) necessary for preparing biological samples have been used in methods development and routine assays. Also, during the first year of the contract, two refrigerated WISPs for the WR 2721 and WR 3689 assays, two Kratos post column reactors for the physostigmine assay, and two refrigerators for the South San Francisco laboratory were purchased. During the second year of the contract, two additional refrigeration units for WISP systems were purchased. An ESA electrochemical detector, the two Kratos reactors and a GC-MS were purchased with funds unrelated to the DAMD17-86-C-6150 contract, but this equipment can be made available, if needed, with DAMD17-86-C-6150 contract funding.

The facilities dedicated for use under this contract encompass rooms 822 and 824 of the Medical Sciences Building and rooms 1257 and 1258 of the Health Science East Building located at the University of California, San Francisco and the off campus laboratory at 296 Lawrence Dr., South San Francisco. The facilities occupy 3900 sq. ft. of space.

Data for an analysis is obtained by comparison of the results for a sample with the results for a series of standard curve samples. The standard curve is constructed by finding the best fit straight line with linear regression analysis of the peak height ratio of the drug to an internal standard versus the spiked concentration of prepared samples of the drug in biological samples. The parameters of the standard curve are used to convert the peak height ratio of the drug peak to the internal standard peak in a chromatogram to the drug concentration of the clinical specimen.

Methodologies for seven drugs were under development during the second year of the contract. Analytical methodologies that were under development in this laboratory are for the determination of the concentrations of the candidate anti-irradiation chemicals, WR 2721 and WR 3689 including WR 225,591 (the thiol of WR3689), in plasma; the anti-cholinesterase drug, pyridostigmine in urine; the anti-malarial drug, mefloquine and its carboxyl metabolite (WR 160,972) in plasma; the antileishmanial compound, WR 6026 in blood to 10 ng/ml and in plasma to one ng/ml; the antimalarial compound halofantrine; and the anti-cholinesterase drug, physostigmine (and its metabolite eseroline) in plasma.

Routine assays for four studies were begun during the second year of the contract. The routine analysis of 781 plasma samples obtained from the Clinical Research Ward of the United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD, for mefloquine under the study titles "The Treatment of *P. falciparum* Malaria with a Combination of Quinine and Tetracycline," "The Treatment of *P. falciparum* Malaria with a Combination of Quinine and Doxycycline," "The Comparison of Chloroquine, Chloroquine-Fansidar, and

Mefloquine as Clinical Prophylactic Agents for *P. falciparum* and *P. vivax* Malaria in Thai Gem Miners Along the Thai-Kampuchean Border," "The Treatment of *P. falciparum* Malaria with WR 180,409, a Pyridinemethanol," "The Treatment of *P. falciparum* Malaria with Halofantrine, a Phenanthrenemethanol," and "The Comparison of Mefloquine and Doxycycline Clinical Prophylactic Agents for Falciparum and Vivax Malaria in Royal Thai Army Troops Assigned to the Thai-Kampuchean Border" has been completed. Routine analysis of 594 samples from the Division of Clinical Pharmacology, Johns Hopkins University School of Medicine was completed for pyridostigmine in plasma, urine, and infusates under the study title "Pharmacokinetics and Pharmacodynamics of Sustained, Low-dose, Intravenous Infusions of Pyridostigmine". In addition, the analysis of samples for determination of the pyridostigmine concentrations was begun for the studies "Comparative Oral Bioavailability Studies of Two Wax Matrix Formulations of Pyridostigmine Bromide in Male Beagle Dogs" and "Safety, Tolerance, Pharmacokinetics and Pharmacodynamics of Single Oral Doses of Sustained-release Pyridostigmine in Healthy Men".

## DOCUMENTATION

### Submittals Related to Contract DAMD17-86-C-6150 for 1987-8

#### Annual Reports

- No. 1: covering 1986-7, dated Apr 13, 1987
- No. 1, revised, dated June 11, 1987

#### Project Status Reports

- No. 5, dated June 26, 1987
- No. 6, dated Sept. 30, 1987
- No. 7, dated Dec. 23, 1987
- No. 8, dated Mar. 30, 1988

#### Study Reports

- No. 6: Mefloquine in plasma, dated May 4, 1987
- No. 6B, revised Oct. 19, 1987
- No. 6C, revised Jan. 8, 1988
- No. 7: Pyridostigmine in urine, dated May 12, 1987
- No. 7B, revised Jan. 12, 1988
- No. 8: Physostigmine in plasma, dated Oct. 20, 1987

#### Analysis Reports

- Mef/P 87-1: Mefloquine in plasma, dated Sept. 16, 1987
- Mef/P 87-1B, revised Feb. 25, 1988
- Pyr/PU 87-2: Pyridostigmine in plasma and urine, dated Jan 27, 1988
- Pyr/PU 87-2B, revised Feb. 24, 1988

## Publications

### Published papers

Ion-paired Liquid Chromatographic Method for the Analysis of Pyridostigmine in Plasma, *Journal of Liquid Chromatography* 10(10), 2231-2246, 1987.

### Submitted papers

Ion-paired Liquid Chromatographic Method for the Analysis of Blood and Plasma for the Antimalarial Drug Halofantrine and its Putative Mono-debutylated Metabolite, accepted for publication in *Journal of Chromatography, Biomedical Applications*.

### Planned papers

Ion-paired Liquid Chromatographic Method for the Analysis of Urine for Pyridostigmine.

## STATUS OF ACCOMPLISHMENTS

The following review summarizes progress made on each of the methodologies under development and describes the routine analyses initiated during the second year of the contract.

### WR 2721 and WR 3689 Studies

High performance liquid chromatographic assays for WR 2721 and WR 3689 in plasma are under development. Briefly, plasma proteins are precipitated with CH<sub>3</sub>CN before sample injection onto a 5  $\mu$ m CN column and drug identification and quantitation is made with the aid of an electrochemical detector. Due to the instability of WR 2721 and WR 3689 at room temperature combined with problems associated with the electro-chemical detector (the detector has a short life and requires considerable attention; the gold/mercury amalgam needs to be refilled every two weeks, then the detector has to be recalibrated), progress towards completion of the analytical methodology required extra time and effort.

An aqueous mobile phase similar to the one used by Shaw and Bonner (1% MeOH, 0.1 M monochloroacetic acid and 10.7 mM ethylamine) was tested to avoid interferences caused by biological samples. The effect of different components of the mobile phase on retention time was tested in a C18 and a CN column. The internal standard for use in the assay of WR 3689 is WR 5144, since WR 80855 has the same retention time as WR 3689. For WR 2721, the internal standard is WR 80855. Each column has its drawbacks. With the C18 column, it was the tailing effect that occurs

in the drug peak after about 2 weeks of continuous use. With the Cyano (CN) column, it was the increase in the retention time of the WR 3689 peak, about 30% late after about 3 weeks of continuous use, which causes the peak to broaden. For the time being, the CN column was chosen for validation studies.

As of September, the WR 3689 method required a 4.6 x 250 mm, 5  $\mu$ m CN column; a 1% MeOH, 0.02 M monochloroacetic acid, and 2 mM sodium octane sulfate mobile phase with a 1.4 ml/min flow rate; and detector settings of +0.3 V and 5 nA. As of September, the WR 2721 method required a 4.6 x 250 mm, 5  $\mu$ m CN column; a 1% MeOH, 0.0125 M monochloroacetic acid, and 2 mM sodium octane sulfate mobile phase with a 1.0 ml/min flow rate; and detector settings of +0.3 V and 5 nA. The validation of the WR 3689 and WR 2721 methods started in September 1987. The completion of the validation has been hindered because of problems with the Hg-Au working electrode. Although every effort was taken to make the gold surface as smooth as possible before a thin film of mercury was placed over the gold surface, a visual inspection of a newly polished working electrode showed an insufficiently smooth mercury surface.

The mobile phase concentration of monochloroacetic acid was changed in December from 0.02 M to 0.0125 M for the WR 3689 method and from 0.0125 M to 0.008 M in the WR 2721 method to avoid an interfering peak that persisted even in a washed column. Validation of the methodologies for both drugs continued. The standard curve concentrations for both compounds ranges from 0.1 to 40  $\mu$ g/ml. The method of weighted least squares is used to calculate the best fit straight line for the standard curve, where the weights  $w = \frac{1}{\text{peak height ratio}^2}$ . Precision and recovery data has been obtained for both compounds over the range 0.2 to 20  $\mu$ g/ml. Stability data for concentrations in the range 0.4 to 19  $\mu$ g/ml has been obtained up to the second month for WR 3689 and will soon be obtained for the first month for WR 2721.

In addition, we have purchased a new electrochemical detector with a glassy carbon electrode (ESA Co., Bedford, MA) in place of the gold/mercury amalgam electrode. We expect to obtain coulometric data with this detector and to test the new system in the near future.

### **Mefloquine Study**

Draft Study Report No. 6 "High Performance Liquid Chromatography (HPLC) of Mefloquine in Plasma" was submitted for approval on May 4, 1987. Revised submittals were made on Oct. 19, 1987 and Jan. 8, 1988.

### **Mefloquine Routine Analysis**

The results of the routine analysis of 781 plasma samples were issued with Analysis Report No. Mef/P 87-1 which was dated Sept. 16, 1987 and was revised Feb. 28, 1988.

### **Halofantrine Study**

A study of the stability of halofantrine in plasma and blood at -80°C has been completed. The study contains data covering a period of four months and provides additional information for Study Report No. 4, dated Aug. 23, 1985 and titled "Ion-Paired Liquid Chromatographic Method for the Analysis of Halofantrine (WR 171,669) and its Putative Metabolite (WR 178,460) in Blood and Plasma". The results were presented in Project Status Report No. 7, dated Dec. 23, 1987.

### **Pyridostigmine in Urine Study**

Draft Study Report No. 7 "High Performance Liquid Chromatography (HPLC) of Pyridostigmine in Urine" was submitted for approval on May 12, 1987 and was revised January 12, 1988.

### **Pyridostigmine in Plasma Study**

Unknown samples for a comparability study of pyridostigmine in plasma analyses have been run and results have been reported by phone.

### **Pyridostigmine in Plasma and Urine Routine Analyses**

The results of the routine analysis of 498 plasma, 72 urine and 24 drug infusate samples were issued with Analysis Report No. Pyr/PU 87-2 which was submitted January 27, 1988 and was revised Feb. 24, 1988.

Routine analyses of 341 dog plasma samples to be submitted in Analysis Report Pyr/P 88-1 and 480 human plasma samples to be submitted in Analysis Report Pyr/P 88-2 were begun during the second year of the contract.

### **Physostigmine Study**

Draft Study Report No. 8 "High Performance Liquid Chromatography (HPLC) of Physostigmine in Plasma" was submitted for approval on Oct. 20, 1987. The assay is linear ( $r^2 = 0.9985$  for a representative standard curve) for physostigmine concentrations in the range 0 to 25 ng/ml with a minimum detection limit of 1 ng/ml using a UV detection method. Recovery of physostigmine from plasma averaged 90.5% (79.0 to 114%) at four concentrations in the assay's linear range. Precision, measured as the coefficient of variation ( $n = 6$ ), ranged from 2.98 to 13.3% for intraday analyses and from 3.20 to 7.67 for interday analyses at four concentrations in the linear range. Accuracy of the method, measured as bias of the measured level ( $n = 6$ ) versus the spiked level, ranged from 2.6 to 6.4% at four concentrations in the assay's linear range. Stability of physostigmine in plasma samples stored at -20°C for a period up to 48 days was demonstrated at four concentrations in the assay's linear range.

The next step in development of the physostigmine assay is to develop an assay to detect and to determine the concentration of eseroline, a physostigmine metabolite, in plasma. This procedure should demonstrate that eseroline does not interfere with the physostigmine measurement. The sensitivity limit for both compounds is targeted in the 0.1 to 0.2 ng/ml range, which is consistent with research of the literature.

Two approaches to the detection of physostigmine and eseroline are being considered. One is to use a fluorescence detector and sample clean up by extraction of the samples. The other is to use an electrochemical detector (ECD) with a Bond Elut sample clean up procedure. The fluorescence detector and extraction clean up method has been successfully used to detect the drug and its metabolite down to the 0.2 ng/ml level. The electrochemical detector with Bond Elut sample clean up approach focuses on finding the proper conditions for the elution of the sample from the Bond Elut. The biggest problem is to stabilize the metabolite during the purification step. The advantage of the ECD approach is that a perfected method can be 2 to 5 times more sensitive than a fluorescence detection method and 10 fold more sensitive than the current UV method. The current elution solution, when adjusted to about pH 3, has been used to construct a sufficiently linear standard curve in the 0.1 to 6 ng/ml range using fluorescence detection.

Noticeable degradation of the metabolite was observed in samples that remained on the WISP sample tray; temperatures in the WISP sample tray sometime reach 35°C. Samples that were refrigerated for 24 hours showed no reduction in eseroline concentration. Therefore, we requested funds for the purchase of two cooling units to upgrade our capabilities.

An SOP for shipment and handling of physostigmine plasma samples was enclosed with Project Status Report No. 7; and an updated SOP for shipment and handling of physostigmine and eseroline plasma samples was submitted on Jan 29, 1988. Blind sample results were enclosed with Project Status Report No. 8.

Study Report Number 9 describing the methodology for analysis of 0.5 ml plasma samples for the determination of concentrations of physostigmine and eseroline, its metabolite, in the range 0.100 to 6.00 ng/ml and including results of blind sample analysis is currently being written. The third month of the stability study has been completed. No further laboratory work is planned at this time.

#### **WR 6026 in Blood and Plasma Study**

The validation (precision and recovery) of an assay for WR 6026 in blood was completed and a methodology was submitted Aug. 24, 1987 that included results of analysis of blood samples originally reported in Analysis Report AY 86-2-A and B (these results were deleted from later revisions of Analysis Report AY 86-2). Inter- and intraday precision analysis of replicate (n=6) spiked samples resulted in coefficients of variation of less than 10% at three concentrations in the range 12.9 to

64.4 ng/ml. The standard curve was linear in the range 6.44 to 96.6 ng/ml with a coefficient of determination ( $r^2$ ) of 0.9981. A study report is in preparation on the validation studies that were run on the assay for WR 6026 in blood. In order to promote greater efficiency in completion of reports we would like to combine the blood and plasma reports into one. Attempts to get the detection limit down to 1 ng/ml in plasma have been focused on altering the mobile phase and the extraction method and using a silica column and a fluorescence detector. This work will not have a very high priority compared to other projects that are currently underway unless we are instructed otherwise.

Blind samples of whole blood spiked with WR 6026 received from WRAIR in early March are currently being analyzed.

### TESTS

The following tests are conducted for the validation of a methodology under development. The sensitivity of the method is demonstrated by the analysis of prepared samples spiked at the drug concentration of the low point of the standard curve. The accuracy of the method is demonstrated by the analysis of blind samples provided by the US government. The reproducibility of the method is demonstrated by interday and intraday analysis of prepared replicate samples spiked at several concentrations. The recovery of the method is determined by comparison of the analyzed concentration of the drug spiked in water versus the analyzed concentration of the drug spiked in biological specimens. The stability of the drug in specimens is determined by analysis of samples that have been frozen for various lengths of time.